A 100% Water Mobile Phase HPLC-PDA Analysis of Selected Neonicotinoid Insecticides

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ABSTRACT

This paper describes a reserved-phase HPLC method for detecting frequently-used neonicotinoid insecticides, acetamiprid (ATP) and imidacloprid (ICP), using an isocratic 100 % water mobile phase. Chromatographic separations were performed an Inertsil® WP300 C4 with water mobile phase and a photodiode-array detector. The total run time was < 7 min. The system suitability was well within the international acceptance criteria. The detection limits were 0.013 ε g ml^{-1} for ATP and 0.015 ε g ml^{-1} for ICP, respectively. A harmless HPLC method for simultaneous detecting ATP and ICP was developed and may be further applied to the quantification in foods.

Keywords

Internal harmonized analytical method; Acetamiprid; Imidacloprid; High-Performance Liquid Chromatography; Photo-diode array

1 INTRODUCTION

Neonicotinoids are a class of neuro-active/systemic insecticides that act on certain kinds of receptors in the nerve synapse, like nicotine, and are used worldwide as agricultural crop protection and environmental pest management, and control fleas on domestic animals [1]: they are registered in > 120 countries and represented 24 % of the global market for insecticides in 2008 (made up 80 % of all seed treatment sales)[2]. One thing that has made neonicotinoid insecticides popular in pest control is their water solubility, which allows them to be applied to soil and be taken up by plants.

In the early 2000s some kinds of neonicotinoids began to come under increasing scrutiny over potential environmental impacts. The use of neonicotinoids was linked in a range of studies to a number of adverse ecological effects, including honey-bee colony collapse disorder and loss of birds due to reduction in insect populations. Increased scrutiny eventually led to restrictions and bans on the use of different neonicotinoids in several countries [3-7].

In December 2013, two neonicotinoid insecticides, acetamiprid (ATP) and imidacloprid (ICP), may affect the developing human nervous system, disclose the European Food Safety Authority (EFSA). Experts from the Authority propose that some guidance levels for acceptable exposure to the two neonicotinoids be lowered while further research is carried out to provide more reliable data on so-called developmental neurotoxicity [8].

Under the circumstances mentioned above, hard monitoring for the presents of ATP and ICP in all food crops is, therefore, important means to further elucidate the residue situation in foods and to prevent the exposure of consumers to these pesticides.

Depending on the recent expansion and diversification in the international food trade, the development of international harmonized methods to determine chemical residues in foods is essential to guarantee equitable international trade in these foods and ensure food safety for consumers. Whether in industrial nations or developing countries, an international harmonized method for residue monitoring in foods is urgently ï needed. The optimal harmonized method must be easy-to-use, economical in time and cost, and must cause no harm to the environment and analyst. Although several techniques based on high-performance liquid chromatographic (HPLC) detection have been developed for the monitoring ATP and ICP [9-15], these methods have crucial drawbacks: 1) all of the methods consume large quantities of toxic organic solvents, acetonitrile and/or methanol [16], in the mobile phases. Risk associated with these solvents extend beyond direct implications for the health of humans and wildlife to affect our environment and the ecosystem in which we all reside. Eliminating the use of toxic solvents and reagents is an important goal in terms of environmental conservation, human health and the economy [17,18]; 2) most of the recent methods are based on LC-MS or -MS/MS. The facilities that LC-MS/MS system is available are limited to part of industrial nations because these are hugely expensive, and the methodologies use complex and specific. These are unavailable in a lot of laboratories for routine analysis, particularly in developing countries. No optimal method that satisfies the aforementioned requirements has yet been identified.

As the first examination problem in the establishment of an international harmonized method for the residue monitoring of ATP and ICP, this paper describes an isocratic 100 % water mobile phase HPLC conditions to detect ATP and ICP without the organic solvent/reagent consumption.

2 EXPERIMENTAL

2.1 Chemicals and Reagents

Standards of acetamiprid (ATP) and imidacloprid (ICP) and distilled water (HPLC grade) were purchased from Wako Pure Chem. Ltd. (Osaka, Japan).

2.2 Equipment

The HPLC system, used for method development, included a model PU-980 pump and DG-980-50-degasser (Jasco Corp., Tokyo, Japan) equipped with a model CO-810 column oven (Thosoh Corp., Tokyo, Japan), as well as a model SPD-M10A

$_{VP}$ photodiode-array (PDA) detector (Shimadzu Scientific Instruments, Kyoto, Japan).

The following four types of non-polar sorbent columns (5 εm d_P; 4.6 mm i.d.; 150 mm length) for HPLC analysis were used: Inertsil® ODS-4; Inertsil HILIC (diol); Inertsil WP300 C4; Inertsil TMS (C1) (GL Sciences, Tokyo, Japan). Table 1 lists the particle physical specifications.

2.3 Operating Conditions

The analytical column was an Inertsil WP300 C4 (150 × 4.6 mm, 5 εm) column using an isocratic mobile phase of water at a flow rate of 1.0 ml min^{-1} at 50 ℃. PDA detector was operated at 190 ï 350 nm: the monitoring wavelengths were adjusted to 245 and 269 nm which represent maximums for ATP and ICP, respectively (Fig. 1). The injection volumes were 10 ï 20 εl.

2.4 Preparation of Stock Standards and Working Mixed Solutions

Stock standard solutions of ATP and ICP were prepared by dissolving each compound in water followed by water to a concentration of 100 εg ml^{-1}. Working mixed standard solutions of these two compounds were prepared by suitably diluting the stock solutions with water. These solutions were kept in a refrigerator (5 ℃).

2.5 HPLC Validation

2.5.1 Linearity

The calibration curve was generated by plotting peak areas ranging from 0.025 to 25 εg ml^{-1} versus their concentrations. The linearity was assessed from the linear regression with its correlation coefficient.

2.5.2 Detection limit

The detection limit should correspond to the concentration for which the signal-to-noise ratio. The value was defined as the lowest concentration level resulting in a peak area of three times the baseline noise.

2.5.3 Robustness

Changes of ± 5 % units of the flow rate (1.0 ml min^{-1}) and the column temperature (50℃) were determined. The effect on the peak areas and the validations in the retention times were evaluated.

2.5.4 System suitability test

The HPLC system suitability is an essential parameter of HPLC determination, and it ascertains the strictness of the system used. The suitability was evaluated as the relative standard deviations of peak areas and retention times calculated for 10 replicate injections of a mixed standard solution (0.5 εg ml^{-1}).

3 RESULTS AND DISCUSSION

3.1 Optimum HPLC Conditions

Using four types of non-polar sorbent columns ((a) C18; (b) diol; (c) C4; (d) C1) (Table 1), the author tested to achieve the separation with a 100 % water mobile phase. This study used water as the isocratic mobile phase and examined column temperatures Ó25 ℃, the flow rates Ó0.75 ml min^{-1}, and HPLC retention times Ò15 min (Table 1). Because the HPLC separations were performed serially, the time/run was critical for routine residue monitoring. The short run time not only increased sample throughout for analysis but also affected the method-development time.

The four columns were compared with regard to the separation between ATP and ICP and the sharpness of peaks obtained upon injection of equal amounts. The chromatographic separations within the conditions ranges examined are

also presented in Table 1.

The complete separation of the two compounds and their symmetrical peaks were obtained by a Column-(c) and water mobile phase with column temperature of 50 ℃ and flow rate of 1.0 ml min^{-1}. Fig. 2 displays that the resulting chromatogram obtained from the HPLC. The two target peaks are clearly distinguished at 5.68 and 6.48 min, respectively . The present HPLC-PDA analysis accomplished optimum separation in a short time without the need for a gradient system to improve the separation and pre-column washing after an analysis.

3.2 HPLC Validation

3.2.1 Main validation data

Table 2 summarizes the validation data for the main performance parameters (linearity, range, detection limit, and system suitability). The system suitability values were well within the international acceptance limits [19].

3.2.2 Robustness

Changes of ±5% of the flow rate and the column temperature had no significant effect on the peak areas, whereas the variations in the retention times were obtained with the flow rate and the column temperature. Normal retention times for ATP and ICP were 6.48 and 5.68 min, respectively. At +5 % the flow rate, the theses retention times were decreased, ranging between 4.1 and 5.4 % and at -5 %, the times were increased ranging between 2.2 and 4.0 %. By changing the column temperature by +5 %, decreasing retention times obtained were 1.7 - 4.3 % and at -5%, the times were increased ranging between 2.1 and 3.0 %. During these studies, both target compounds were separated.

4 CONCLUSION

In the present paper, a HPLC-PDA method for detecting ATP and ICP using an isocratic 100 % water mobile phase has been successfully established. The water mobile phase method is harmlessness to the environment and to humans and has a short run time and high system suitability. The HPLC system may be proposed as an international harmonized method for detecting ATP and ICP. For the quantification in various foods, the proposed HPLC method will be applicable enough by performing a suitable sample preparation technique.

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Legends to figures

Fig. 1: Typical absorption spectra of peaks for ATP (dashed line) and ICP (solid line) standards in the HPLC chromatogram.

Fig. 2: Typical chromatograms of a standard mixture (0.5 εg ml^{-1}) obtained from the HPLC system. PDA set at 245 nm (Ch 1) or 269 nm (Ch 2). The injection volume was 15 εl. Peaks, 1= ICP (retention time, R_t= 5.68 min); 2= ATP (R_t= 6.48 min).